



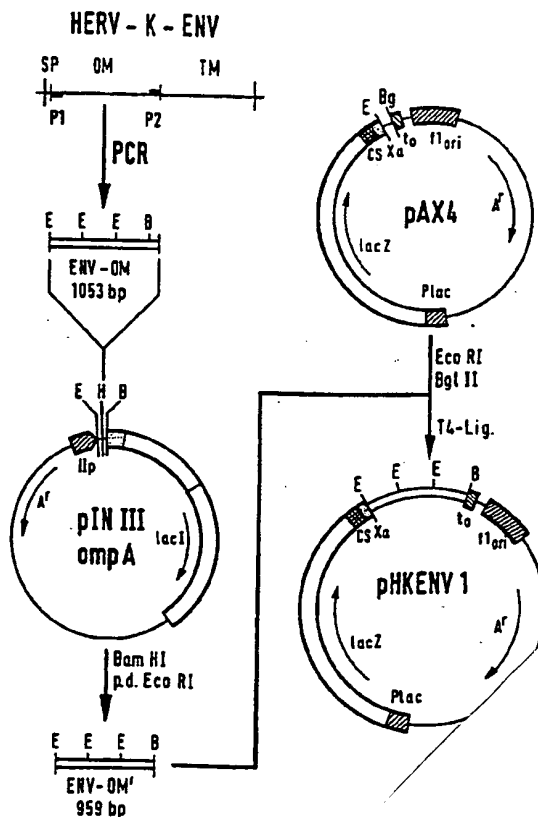
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(54) Title: HUMAN ENDOGENOUS RETROVIRUS-K-ENVELOPE-GENE ENCODED GLYCOPROTEIN, AND A MONOCLONAL ANTIBODY FOR DIAGNOSTIC AND THERAPEUTIC PURPOSES

(57) Abstract

The human endogenous retroviral K (HERV-K) genomes have distinct homologies to murine mammary tumor virus which is involved in the genesis of murine breast cancer. By means of a polymerase chain reaction we amplified a 1053 bp DNA segment (nt.6791-7842) from the putative outer membrane part (nt.6733-7842) of the HERV-K-envelope gene. A 959bp (nt.7691-7749) part of the amplified DNA was inserted into the pAX-bacterial-expression-vector. A recombinant fusion protein of a HERV-K-envelope-portion with β -galactosidase was obtained. A monoclonal antibody was generated which recognizes the envelope portion of the HERV-K-envelope- β -galactosidase-fusion's protein (FP). This mAb is capable of immunoprecipitating a 67kD glycoprotein from the human breast carcinoma cell line T47D, the amount of which is strongly enhanced after stimulation with estradiol/progesterone. The same band could be precipitated from other carcinoma cell lines (Hep2, MCF7 and HE-LA), but not from the human B-lymphoblastoid cell line Raji and the mouse myeloma cell line Ag8.653. We interpret these data to suggest that the antigen recognized by the anti-HERV1 mAb is the translation product of an active HERV-K-env gene or whole HERV-K provirus.



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HUMAN ENDOGENOUS RETROVIRUS-K-ENVELOPE-GENE ENCODED GLYCOPROTEIN, AND A MONOCLONAL ANTIBODY FOR DIAGNOSTIC AND THERAPEUTIC PURPOSES.

In the early forties the murine mammary tumor virus (MMTV) was found to be involved in murine mammary carcinogenesis (Vischer, M. B., Green, R. G. & Bittner, J. D. (1942) Pro.Soc.Exp.Biol.Med. 49, 94-96). By means of hybridization techniques the existence of genomic integrated proviruses could be detected in all strains of mice, in both those with high or low risk of mammary tumor development (Varmus, H.E., Bishop, J. M. Nowinski, R.C. & Sarkar, N.H. (1972) Nature 238, 189-190). This fact suggested that, besides the presence of MMTV-proviruses, other factors also must participate in mammary carcinogenesis, such as, perhaps, the expression of neighbouring genes driven by the MMTV promotor. Even retroviruses lacking viral oncogenes may be involved in tumorigenesis by integration into sites where they can activate transcription of cellular oncogenes (Varmus, H.E. (1982) Cancer Surv. 2, 301). Meanwhile, the protooncogenes c-myc, int-1 and c-neu were described to be possible targets of an insertion of MMTV-provirus resulting in the development of mammary tumors (Sinn, E., Muller, W., Pattengale, P., Tepler, I., Wallace R & Leder, P. (1987) Cell 49, 465-475), (Tsukamoto, A.S., Grosschedl, R., Guzman, R.C., Parslow, T & Varmus, H.E. (1988) Cell 55, 619-625), (Muller, W.J., Sinn, E., Pattengale, P.K., Wallace, R. & Leder, P. (1988) Cell 54, 105-115).

Recently MMTV has been drawn to the attention of immunologists, because it has been found that endogenous MMTV may have a major impact on the formation of the T-cell repertoire. MMTVs are shown to encode for superantigens, which by binding to MHC class II antigens and to the VB-domain of the T-cell receptors (Acha-Orbea, H. & Palmer, E. (1991) Immunol. Today 12, 356-361).

The existence of a human mammary tumor virus has been considered for a long time, but not conclusively proven. In the early seventies the presence of reverse transcriptase activity and "retroviruslike particles" (RLVP) in the human milk samples was described (Schlom, J., Spiegelman, S. & Moore, D. (1971) Nature 231, 97-100). But, until now, there has been no evidence for the infectivity of these particles. RVLP with reverse transcriptase activity were also found in the human breast carcinoma cell line T47D after stimulation with estradiol and progesterone. These particles were shown to contain a glycoprotein gp69 that is recognized by antisera raised against MMTV-gp52 (Ohno, T., Mesa-Tejada, R., Keydar, I., Ramanarayanan, M., Bausch, J. & Spiegelman, S. (1979) Proc.Natl.Acad.Sci.USA 76, 2460-2464), (Segev, N., Hizi, A., Kirenberg, F. & Keydar, I. (1985) Proc.Natl.Acad.Sci.USA 82, 1531-1535).

These publications describe for the first time the detection of a possible retrovirus encoded protein in the human tumor cellline T47d.

Another approach to identify a MMTV-analog in humans was attempted by cloning and characterization of human endogenous retroviral sequences which hybridize with MMTV-probes under low stringency conditions (May, F.B., Westley, B.R. & Rocheford, H. (1983) Nucl. Acids. Res. 11, 4127-4139), (Westley, B. & May, F.E.B. (1984) Gene 28, 221-227), (Deen, K.C. & Sweet, R.W. (1986) J. Virol. 57, 422-432). These publications describe the MMTV-analog in the human genome.

One group of human endogenous retroviral genomes, designated HERV-K genomes (K represents the existence of a primer binding site for a putative tRNA-Lysine primer for reverse transcription), was detected by hybridization with a Syrian hamster A-type particle pol probe. Typical HERV-K-genomes were 9.1 or 9.4 kilobases in length, having long terminal repeats of ca. 970 bp and genes for gag, pol, and env as well as a gene likely to encode for a protease (prt). The env gene is divided into parts putatively encoding for a signal peptide, an outer membrane protein, and a transmembrane protein. The outer membrane part contains 7, the transmembrane part 4 possible glycosylation sites. Sequence comparison of the HERV-K10+ genome revealed distinct homologies between HERV-K and MMTV, especially in the putative envelope-gene-region. In contrast to other human endogenous retroviral genomes, this HERV-K-genome contains large open reading frames in the putative gag, prt, pol and env., region. The HERV-K10+ sequence contains a stop codon at pos. 7210 that is not present in the three other characterized HERV-K-genomes (Ono, M., Yasunaga, T., Miyata, T. & Ushikubo, H. (1986) J. Virol. 60, 589-598).

Thus, it seems possible that a HERV-K-provirus exists which may encode for a complete env protein. It has been calculated by filter hybridizations that around 50 HERV-K-proviruses exist per haploid genome, although it is not known if they are all complete. Furthermore, an 8.8 kb HERV-K-mRNA transcript was detected in estradiol/progesterone treated T47D cells and in other carcinoma cell lines by hybridization with probes from the gag-, pol- and env-gene region of HERV-K10 (Ono. M., Kawakami, M. & Ushikubo, H. (1987) J. Virol. 61, 2059-2062). He describes the DNA-Sequence of the HERV-K retrovirus.

The products of the HERV-K-genes have not yet been characterized, but it has been speculated that a relationship between RVLP and the HERV-K-genomes could perhaps be found (Ono, M., Yasunaga, T., Miyata, T. & Ushikubo, H. (1986) J. Virol. 60, 589-598).

In the present application we describe for the first time the identification of a 67 kD glycoprotein which is detectable in lysates from T47D cells as well as in other human carcinoma cell lines by means of a mAb raised against a recombinant HERV-K-envelope protein representing a large segment of the putative outer membrane protein.

Materials

Restriction enzymes and T4DNA-ligase were purchased from Pharmacia (Uppsala, Sweden), IPTG, DNaseI, lysozyme and BSA from Sigma (St. Louis, USA). FXa and Endoglycosidase F were obtained from Boehringer (Mannheim, Germany). 125 Iodine was purchased from Amersham (Buckinghamshire, UK). The pAX-vector was obtained from Medac (Braunschweig, Germany) and the pINIII-ompA vector was a friendly gift from Dr. W. Schwäble, Mainz, Germany.

CELL LINES

The T47D, MCF7 (both human breast adenocarcinoma), HELA (human squamous cell carcinoma), Hep2 (human laryngeal squamous cell carcinoma), Raji (human B-lymphoblastoid) as well as Ag8.653 (mouse myeloma) cell lines were maintained in RPMI containing 10% FCS. Stimulation of the T47 D cell line with estradiol/ progesterone (Sigma, St.Louis, USA) was done as previously described (Ono. M., Kawakami, M. & Ushikubo, H. (1987) J. Virol. 61, 2059-2062).

ANTIBODIES

BALBc-mice were immunized intraperitoneally three times in intervals of 2 weeks with 10 µg rHERV-env-OM-FP emulgated in complete Freund's adjuvans and finally immunized intrasplenically once with rHERV-env-OM-FP lacking adjuvants. A monoclonal antibody, anti-HERV1, recognizing a recombinant HERV-K-envelope protein was prepared by standard techniques (Fazekas de St. Groth, S. & Scheidegger, D. (1980) J. Immunol.Methods 35, 1-12). This review describes the production of monoclonal antibodies.

Hybridomas were screened by ELISA and confirmed by Western blot using rHERV- nv-OM-FP and control proteins.

Example

PCR AND CLONING PROCEDURE

A standard 100µl amplification reaction vessel containing 5µg T47D genomic DNA, 100pM each primer (5'CAGTCACATGGATGGATAAT, nt.6791-6810, according to (Ono, M., Yasunaga, T., Miyata, T. & Ushikubo, H. (1986) J. Virol. 60, 589-598) and 5'TCTTTTGGATCTATTTAAACACC antisense to nt.7842-7819, same reference as above, 200µM dNTPs, 10µl 10x amplification buffer and 2U Taq polymerase (both Promega, Madison, USA) was amplified in a Bio-med 60 Thermocycler for 25 cycles (92°C 90 sec, 56°C 2 min, 72°C 3 min). The resulting 1053bp DNA fragment was purified by agarose electrophoresis and consecutive electroelution and then ligated into the blunt-ended HindIII site of pINIII-ompA1 (Ghrayeb, J., Kimura, H., Takahara, M., Hsiung, H., Masui, Y. & Inouye, M. (1984) EMBO J. 3, 2437-2442). This publication describes a expression-vector for E. Coli, which is utilized in this invention.

The DNA fragment was then digested with BamHI and partially with EcoRI and after agarose gel purification of the resulting 959 bp fragment ligated into a EcoRI/ BamHI cut pAX-vector (provided by Medac, Braunschweig FRG). Positive recombinants were screened by hybridization with the PCR fragment. The identity of the insert was checked by sequencing of 200bp from the 3'terminus and found to be 99% homologous to the HERF-K-10+sequence. All cloning, hybridization, and sequencing procedures were done according to standard protocols (Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) Molecular cloning Second Edition (Cold Spring Harbour Laboratory Press, New York).

EXPRESSION OF rHERV-env-OM-FP

The pAX-Plasmid containing a HERV-K-envelope insert, designated pHERVKENV-pAX4, was transfected into the E.coli TG2 strain and the expression of rHERV-env-OM-FP was induced by IPTG as described by Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) Molecular Cloning, Second Edition (Cold Spring Harbour Laboratory Press, New York).

The fusion protein was extracted from the inclusion bodies by the following procedure: The bacteria from a IPTG induced 500 ml culture were harvested by centrifugation at 7000g, 4°C and 10 min. The cells were suspended in 5 ml PBS containing 1 mg/ml Lysozym, 1% Triton-X-100, 10µg/ml DNaseI and 15 mM MgCl₂ and incubated 60 minutes on ice. The lysate was then centrifuged at 7000g 15min 4°C. The pellet was suspended in 10 ml 10mM Sodiumtetraborate pH 9.0 containing 0.5 CHAPS and incubated 30 minutes at room temperatur. The lysate was again centrifuged at 7000 g 15 min 4°C and the pellet suspended in 10 ml 50 mM Tris/HCl pH 8.0 containing 8 M Urea, 1 mM EDTA and 0,15 M NaCl and incubated for 30 minutes at room temperatur. After centrifugation at 7000g 15 min 4°C the supernatant containing rHERV-env-OM-FP was dialysed against 50 mM TRIS/Hcl pH 8.0 containing 1M UREA, 1 mM EDTA and 0,1M NaCl and then loaded onto a 20ml DEAE-Trisacryl chromatography column. Washing of the column was done with 500ml of 50mM TRIS/HCl pH 8.0 containig 1M Urea, 1mM EDTA and 100 mM NaCl and rHERV-env-OM-FP was eluted with 50mM TRIS/HCl pH 8.0 containing 1 M UREA, 1 mM EDTA and 0,25M NaCl.

DIGESTION OF rHERV-env-OM-FP WITH ENDOPROTEINASE Xa (FACTOR Xa)

200µg protein from the 8M Urea fraction were loaded onto a SDS-PAGE according to Laemmli (Laemmli, U.K: (1970) Nature 227,680-685). After separation, the band containing rHERV-env-OM-FP was cut from the gel and quenched 16h in 1,5 ml PBS at 4°C. After centrifugation at 1500g for 10min the supernatant was removed and dialysed against FXa cleavage buffer (0,5M Urea, 0,1M NaCl, 1mM CaCl₂, 50mM TRIS pH 8.0) and then transferred to microfuge tubes and incubated with or without 1µg FXa 16h at 18°C (200µl/reaction). The reaction was stopped by addition of SDS-PAGE sample buffer containing 5% β-mercaptoethanol and boiling for 2 min. The samples were analysed by Western blot with mAb anti-HERV1 and a negative control antibody from the same Ig-subclass.

IMMUNOPRECIPITATION

BSA, anti-HERV1 and an anti-tubulin mAb as a negative control were coupled to CNBr-activated Sepharose according to the manufacturers instructions (Pharmacia, Uppsala, Sweden).

Adherent cells (5×10^7 cells) were washed four times with PBS and harvested with 5mM EDTA in PBS with the aid of a rubber policeman. Cells grown in suspension were washed four times with PBS. The cells were centrifuged at 1200g for 10min at 4°C and the cell pellet solubilized 15 min on ice with lysis buffer (0,5% Nonidet P40, 0,5% Triton X-100, 0,5% Deoxycholate, 5mM EDTA, 2mM PMSF in PBS). The lysate was then centrifuged at 15000g for 10min and the supernatant

labeled with iodine-125 by the iodogen/iodobead method according to the manufacturers instructions (Pierce, Oud-Beijerland, Netherlands). Labeled proteins were recovered from free iodine 125 by chromatography on a small Sephadex G 50 column. The lysates were then precleared by incubation with 200µl BSA-sepharose (1h, RT) and then divided into two aliquots and incubated either with 25 µl anti-HERV1-sepharose or with anti-tubulin-sepharose (1h, RT). The sepharoses were washed six times with lysis buffer containing 0,5M NaCl and then SDS-sample buffer containing 5%(V/V) β -mercaptoethanol (50µl) was added and incubated 2 min at 96°C. The supernatant was loaded onto a SDS-PAGE according to Laemmli (Laemmli, U.K: (1970) Nature 227,680-685).

After electrophoresis, the gel was stained with Coomassie-brilliant-blue, dried and exposed to Cronex4 film (Dupont, Bad Homburg, Germany) for 24-48 hrs.

In one experiment iodine-labeled Hep2-lysate was incubated with BSA-sepharose and anti-HERV1-sepharose as above. After 4 times washing with the above lysis buffer with 0,5M NaCl, the sepharose was washed once with PBS containing 0,3% Triton-X100 and 0,1M sodium acetate. The sepharose was divided into two aliquots in 100 μ l of PBS with 0,3% Triton-X100 and 0,1M sodium acetate and incubated with or without 0,5U Endoglycosidase F 16h at RT. Washing was again done twice with lysis buffer with 0,5M NaCl. The samples were then analysed by SDS-PAGE and autoradiography as above.

Results

PCR AND CLONING OF A HERV-K ENVELOPE GENE FRAGMENT

A 1053 bp DNA fragment (nt.6791-7842, ref.14) corresponding to the majority of the putative outer membrane env fragment (nt.6733-7842, ref. (Ono, M., Yasunaga, T., Miyata, T. & Ushikubo, H. (1986) J. Virol. 60, 589-598) of HERV-K10+ was amplified by PCR using T47D genomic DNA as a template and an oligonucleotide -primer-pair synthesized according to the published HERV-K10+ sequence. This fragment was ligated into the pINIII-ompA1 vector and subsequently digested with BamHI and partially with EcoRI. A 959bp fragment (pos.6791-7749, ref. Ono, M., Yasunaga, T., Miyata, T. & Ushikubo, H. (1986) J. Virol. 60, 589-598) containing the EcoRI-site from the pINIII-ompA1-vector on its 5'terminus was isolated and then ligated into an EcoRI/BglIII cut pAX-expression vector. This vector allows the production of a beta-galactosidase FP which should contain a recombinant HERV-env-OM (fig.1). The identity of the insert as a HERV-K-envelope gene was checked by partial sequence analysis. 200bp were sequenced on the 3'terminus and found to be identical with the sequence of HERV-K10+ except for two A to T substitutions at nt. 7657 and 7691 (Ono, M., Yasunaga, T., Miyata, T. & Ushikubo, H. (1986) J. Virol. 60, 589-598), which results in a substitution of Ser instead of Thr and Val instead of Asp, respectively. However, as found by restriction enzyme digestion, our cloned insert contains two EcoRI restriction sites which are not present in the published HERV-K10+ sequence and which are not located inside the 200 bp at the 3'terminus.

Figure 1 shows the cloning procedure schematically.

GENERATION OF rHERV-env-OM-FP

After induction of FP synthesis with IPTG, the inclusion bodies were prepared from the bacteria and then fractionally solubilized. The rHERV-env-OM-FP was found to be extractible in the presence of 8 M UREA. As determined by SDS-PAGE, rHERV-env-OM-FP has a m.w. of 155 kD which is composed of a 118kD β -galactosidase portion and a 37kD HERV-K-envelope-outer-membrane-protein portion which is the expected size for complete translation of the 959bp insert.

PRODUCTION OF A MONOCLONAL ANTIBODY REACTING WITH THE ENVELOPE PROTEIN PORTION OF rHERV-env-OM-FP

One monoclonal antibody (anti-HERV1) recognizing the HERV-K-envelope-protein portion of rHERV-env-OM-FP was obtained after immunization of BALBc mice with rHERV-env-OM-FP and generation of hybridomas. This mAb exhibited reactivity with rHERV-env-OM-FP but lacked reactivity with β -galactosidase. Other bacterial proteins extracted from IPTG-induced E.coli transfected with the plasmid pAX without insert were also not recognized.

Furthermore, anti-HERV1 recognized the 37kD envelope protein after cleavage of rHERV-env-OM-FP with endoproteinase Xa.

By means of an ELISA this mAb has been identified as a mouse IgM mAb.

IMMUNOPRECIPITATION OF A 67KD GLYCOPROTEIN FROM HUMAN CARCINOMA CELL LINES

The human breast carcinoma cell line T47D, either untreated or after stimulation with estradiol/progesterone, as well as the Hep2 (human laryngeal carcinoma), HELA (human squamous cell carcinoma), MCF7 (human breast carcinoma), Raji (human B-lymphoblastoid) and the Ag8.653 (mouse myeloma) cell lines were lysed and the immunoprecipitated with the anti-HERV1 mAb. From all these cell lines, with exception of Raji and Ag8.653 cells, a 67kD protein could be precipitated with the anti-HERV1 mAb, but not with a control mAb from the same Ig-subclass (IgM). Stimulation of T47D with estradiol/progesterone strongly enhanced the expression of the 67kD protein

Treatment of the 67 kD-protein with endoproteinase F reduced the m. w. to 58-60kD, indicating the presence of N-linked carbohydrate moieties of at least 7-9kD

In the present report, we describe the detection of a 67kD antigen in the estradiol/progesterone treated human breast carcinoma cell line T47D and in the carcinoma cell lines HELA, Hep2 and MCF-7 by a mAb raised against a recombinant protein encoded by the outer membrane part of a HERV-K-env gene.

For the generation of a recombinant HERV-K-env-outer-membrane protein (rHERV-env-OM-FP) we used the pAX-expression-vector system, because it allows high level expression of a β -galactosidase-FP in which the β -galactosidase part and the foreign-protein portion are separated by a collagen "spacer" that facilitates independent folding of the foreign protein during its syntheses. A further advantage is that the FP could be cleaved by FXa into a β -galactosidase-collagen-spacer protein and the foreign protein. Our insert is slightly different from the published HERV-K10+ sequence, as it contains 2 EcoRI sites which are not present in HERV-K10+ and at least in the 200th base-position from the 3'-terminus.

(Ono, M., Yasunaga, T., Miyata, T. & Ushikubo, H. (1986) J. Virol. 60, 589-598).

The env part of rHERV-env-OM-FP has a m. w. of 37kD, indicating that the 959bp insert was fully translated without internal termination. This protein represents the translational product of nt. 6791 to 7749, thus, 86,5% of the whole outer membrane portion (nr.6733-7842, ref. (Ono, M., Yasunaga, T., Miyata, T. & Ushikubo, H. (1986) J. Virol. 60, 589-598) of the HERV-K10-env gene. It seems likely that that a complete outer membrane protein has

a peptid core of approximately 43KD and carbohydrate moieties of 25 kd since the outer membrane env gene of HERV-K10+ contains 7 possible glycosilation sites. By treatment with Endoglycosidase F we could reduce the molecular weight of the 67kd protein to about 59kd. However, it seems possible that the gp67 is not completely deglycosilated, since the Endoglycosidase F treatment was performed on an immobilized antibody/antigen complex.

T47D cells, established from pleural effusion from a human breast cancerpatient, have receptors for estrogen, progesterone and androgen (Keydar, I., Chen, L. Karby, S., Weiss, F.D., Delarea, J., Radu, M. Chaitchek, S. & Brenner, H.J. (1979) Eur. J. Cancer 15, 659-670).

In these cells HERV-K-mRNA transcripts had already been detected, but only at a basal level when the cells were kept in normal tissue culture medium supplemented with 10%FCS. This expression could be increased when the cells were treated with 10nM estradiol on day 1 and 100nM progesterone on day 2. mRNA-transcripts for HERV-K have also been described in Hep 2 and HELA cells, whereas the MCF-7-cell line has not been examined (Ono. M., Kawakami, M. & Ushikubo, H. (1987) J. Virol. 61, 2059-2062).

This is in line with our description of the distribution of the 67kd antigen, characterized by our anti-HERV1.mAb. The expression of our 67kd antigen in T47D was also up regulated by the combination of estradiol and progesterone.

Particles containing "reverse transcriptase activity" as well as ther structural features of retr viruses and therefore term d RVLP are released from T 47D cells depending upon stimulati n with estradiol

followed by progest one. One antigen associated with PVLP, gp68, has been identified to be recognized by a polyclonal antibody raised against the MMTV-env-gp52 (Segev, N., Hizi, A., Kirenberg, F. & Keydar, I., (1985) Proc.Natl.Acad.Sci.USA 82, 1531-1535).

A mAb (H23) has been raised against T47D-RVLP which was found to recognize a 68kD glycoprotein associated with T47D-RVLP. In the same study (Keydar, I., Chou, C.S., Hareuveni, M., Tsarfaty, I., Sahar, E. Selzer, G., Chaitchik, S. & Hizi, A. (1989)

Proc.Natl.Acad.Sci.USA 86, 1362-1366) it was speculated that the gp68 recognized by the anti-MMTV-gp52-antisera and the gp68 detected by H23 are very similar and possibly identical. In this publication, the cell-line T47D are described in detail. In contrast to those findings, a later study revealed that the H23 mAb recognized two high molecular mass proteins (>200kD) from T47D cell extracts and culture supernatants. Furthermore, it was found that H23 did not recognize a MMTV protein which is explained by an only restricted similarity of a few epitopes between MMTV and T47D-RVLP. Sequences derived from cDNA clones isolated by immunoscreening with the H23 mAb revealed that H23 recognizes a mucin-like epithelial tumor antigen. However, the H23 mAb demonstrated a high specificity for breast carcinoma tissue,

but it has also been detected on the surface of T47D cells as well as in culture supernatants and in pleural effusion fluids from breast cancer patients.

We assume that our gp67, which is characterized by the anti-HERV1-mAb, is located mainly intracellular, since we could not precipitate labeled antigen from surface-labeled cells. Furthermore, the antigen was not detected by immunoprecipitation with our mAb in a pleural effusion fluid of a patient with breast cancer. The detection of this protein by indirect immunofluorescence and immunohistochemistry has also been unsuccessful. One possible explanation could be that the epitope of anti-HERV1 is blocked by an associated, retrovirally or chromosomally encoded protein and only lysis, as done by the immunoprecipitation experiments, liberated the site recognized by our mAb. Some immunofluorescence or immunohistochemistry fixation procedures also result in a high unspecific background when an IgM mAb is used. Several facts support the hypothesis of Ono, M. et al, that RVLP or at least the RVLPgp68, recognized by anti-MMTV-gp52, may be encoded by an active HERV-K-provirus:

- (a) HERV-K-genomes have (especially in the env-region) significant relationship to MMTV with respect to their deduced amino acids and structure,

- (b) antibodies to MMTV have been shown to crossreact with T47D-RVLP
- (c) transcription of HERV-K-genomes as well as the production of RVLP are induced on T47D cells by treatment with estradiol and progesterone and
- (d) our description of a 67kD antigen that possibly is related or identical to the gp68 recognized by anti-MMTV-gp52 and that is recognized by a mAb established against a recombinant HERV-K-env-outer-membrane-protein.

During biosynthesis the precursor protein may be split into an outer membrane glycoprotein and a transmembrane glycoprotein by a virus-encoded protease, as it has been demonstrated for many retroviruses including MMTV and HIV.

The H23 epitope seems to be restricted to breast carcinoma tissue, whereas gp67 is also found in carcinoma cells of other than breast origin. The presence of H23-antigen in these cell lines has not yet been described in the literature. The breast carcinoma specificity of the H23 mAb may be due to an epitope variability of the broader distributed epithelial tumor antigen.

Finally, it seems likely that different HERV-K-proviruses encode for env-glycoproteins which may differ from each other in respect to cellular location, distribution, and reactivity with distinct mAbs.

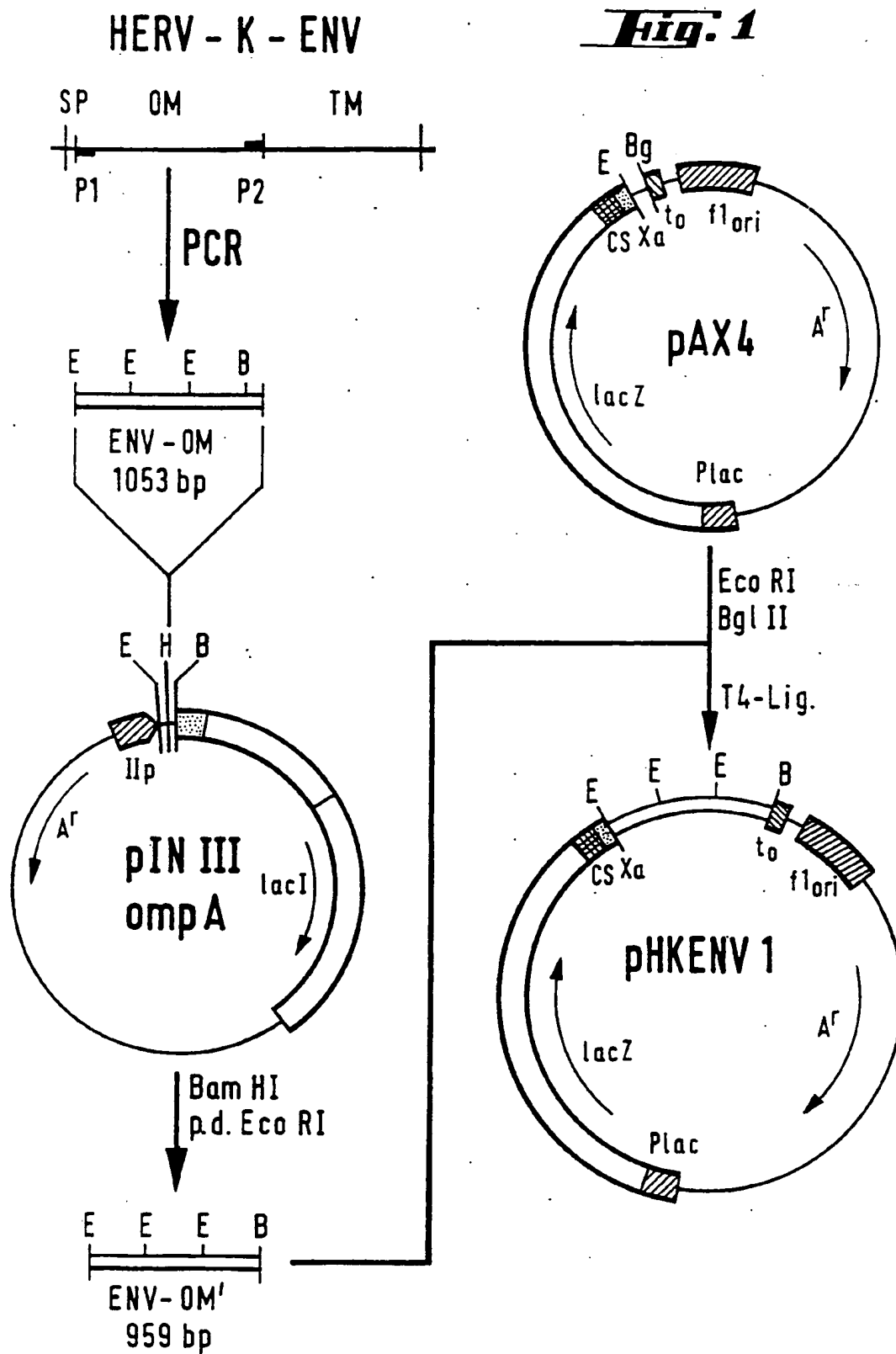
ABBREVIATIONS

BSA	bovine serum albumine
ELISA	enzyme linked sorbent assay
FP	fusion protein
FXa	endoproteinase Xa (blood coagulation factor Xa)
HERV	human endogenous retrovirus
IPTG	Isopropyl- β -D-thiogalactopyranoside
mAb	monoclonal antibody
MMTV	murine mammary tumor virus
nt.	nucleotide position
PAGE	polyacrylamid-gel electrophoresis
PBS	phosphate buffered saline
PCR	polymerase chain reaction
RVLP	retrovirus-like-particles
RNA	ribonucleic acid
tRNA	transfer ribonucleic acid
Ig	Immunoglobulin

Claims

1. Monoclonal antibody, characterized in that it is specific versus a glycoprotein of human tumor cells.
2. Glycoprotein or protein, characterized in that it is encoded by a human endogenous retrovirus K-envelope-gene.
3. Process for the production of a protein or a glycoprotein according to claim 2, characterized in that a pax-vector is expressed and hybridized according to per se known methods, the resulting plasmid is transfected to E.coli TG2-strain and the protein is expressed by the bacterium and isolated.
4. Use of the monoclonal antibody, according to claim 1, for the diagnosis and treatment of cancer.

5. Medicament for the treatment of Cancer, containing a monoclonal antibody according to claim 1 and the usual fillers and auxiliaries.
6. Process for producing a medicament according to claim 5, characterized in that the monoclonal antibody is processed in the usual way with conventional fillers and auxiliaries.
7. A monoclonal antibody as claimed in claim 1 substantially as hereinbefore described with reference to the examples.
8. A glycoprotein or protein as claimed in claim 2 substantially as hereinbefore described with reference to the examples.
9. A process as claimed in claim 3 substantially as hereinbefore described with reference to the examples.



INTERNATIONAL SEARCH REPORT

Intern. Application No
PCT/EP 93/02723

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/48 C07K15/28 A61K39/00 G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 86 , February 1989 , WASHINGTON US pages 1362 - 1366 KEYDAR, I. ET AL. 'Production and characterization of monoclonal antibodies identifying breast tumor-associated antigens' cited in the application see page 1363 - page 1364; figures 3-4; table 1</p> <p style="text-align: center;">--- -/-</p>	1,4-7

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

6 January 1994

Date of mailing of the international search report

28 -01- 1994

Name and mailing address of the ISA

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Authorized officer

Espen, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 93/02723

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA vol. 82 , March 1985 , WASHINGTON US pages 1531 - 1535 SEGEV, N. ET AL. 'Characterization of a protein, released by the T47D cell line, immunologically related to the major envelope protein of mouse mammary tumor virus' cited in the application see page 1533 - page 1534 ----</p>	2,8
Y	<p>JOURNAL OF VIROLOGY vol. 60, no. 2 , November 1986 pages 589 - 598 MASAO ONO ET AL. 'Nucleotide sequence of human endogenous retrovirus genome related to the mouse mammary tumor virus genome' cited in the application see page 591; figures 1,2 ----</p>	2,3,9
Y	<p>GENE vol. 93 , 1990 , AMSTERDAM NL pages 129 - 134 MARKMEYER, P. ET AL. 'The pAX plasmids: new gene-fusion vectors for sequencing, mutagenesis and expression of proteins in Escherichia coli' see the whole document ----</p>	2,3,9
A	<p>GENE vol. 121 , 2 November 1992 , AMSTERDAM NL pages 181 - 182 ZUECO, J. & A. BOYD 'Protein A fusion vectors for use in combination with pEX vectors in the production and affinity purification of specific antibodies' see figure 1 -----</p>	2,3,9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 93/02723

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claim 4 is directed to a method of treatment and to a diagnostic method as far as practised on the human/animal body the search has been carried out and based on the alleged effects of the compound/ composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.